

1/30

Sal I    Bam H I

|    |  
----- - pUC 19

Xba I

1 ATGGAGTCAA AGTTTGCTCA CATCATTGTT TTCTTTCTTC TTGCAACTTC  
original sequence - ag a  
51 CTTTGAAACT CTCTTGGCAC GAAAAGAAAG Tgatggacca gagatcttag  
mutagenic primer  
101 aacTTC AAAA GGAATTGAA TGCAATGGAA AACAAAGGTG GCCAGAACTT  
151 ATTGGTGTAC CAACAAAGCT TGCTAAGGGG ATAATTGAGA AGGAAATTC  
201 ACTCATAACT AATGTTGAGA TACTACTGAA TGGTTCCTCCA GTCACAATGG  
251 ATTATCGTTG TAATCGAGTT CGTCTTTTTC ATAACATTTT GGGTGATGTT  
301 GTACAAATTC CTAGGGTGGC TTAA

Figure 1

1 GAATTCGCA AGGA~~gcacac~~ ~~ccggctgttc~~ ~~acctg~~CTGCA GAGATGGTGC  
upstream primer  
51 ACGCAAACCTC CCCGCTGCTG CTGCTGCTGC TGCTCAGCCT GGCTCTGGT~~g~~  
cc t- original sequence  
101 ~~gtcccgga~~ ~~tccttaccac~~ AAAGTGCTCG CTGACTGGGA AATGGACCAA  
mutagenic primer  
151 CGATCTGGGC TCCAACATGA CCATCGGGGC TGTGAACAGC AGAGGTGAAT  
201 TCACAGGCAC CTACATCACA GCGTAACAG CCACATCAAA TGAGATCAAA  
251 GAGTCACCAC TGCATGGGAC ACAAACACC ATCAACAAGA GGACCCAGCC  
301 CACCTTTGGC TTCACCGTCA ATTGGAAGTT TTCAGAGTCC ACCACTGTCT  
351 TCACGGGCCA GTGCTTCATA GACAGGAATG GGAAGGAGGT CCTGAAGACC  
401 ATGTGGCTGC TGCGGTCAAG TGTTAATGAC ATTGGTGATG ACTGGAAGAGC  
451 TACCAGGGTC GGCATCAACA TCTTCACTCG CCTGCGCACA CAGAAGGAGT  
501 GAGGATGGCC CCGCAAAGCC AGCAACAATG CCGSAGTGCT GACACTGCTT  
! Hind III  
551 GTGATATTCC TCCCCAATAA AGCTTG

Figure 2

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EcoR I  
 1 GAATTTCGCAT ATGGCTGAAG CTGGTATCAC CGGTACTTGG TACAACCAGC  
 51 TGGGGCTCTAC CTTCATCGTT ACCGCTGGTG CTGACCGTGC ACTGACCGGT  
 101 ACTTACGAAA GCGCTGTTGG TAACGCTGAA AGCCGTTATG TTCTGACCGG  
 151 TCGTTACGAC TCTGCTCCGG CTACCGACGG TTCTGCTACT GCTCTGGGTT  
 201 GGACCGTTGC TTGGA AAAAC AACTACCGTA ACGCTCACTC TGCTACCACC  
 251 TGGTCTGGCC AGTACGTTGG TGGTGTGAA GCTCGTATCA ACACCCAGTG  
 301 GCTGCTGACC TCTGCTACCA CCGAAGCTAA CGCTTGGAAA TCTACCCGTG  
 351 TTGGTCACGA CACGTTACCC AAAGTTAAAC CGTCTGCTGC TTCTATCTAGA  
 Xba I

Figure 3

Sal I altered Bam H I\*

----- pUC 19

Xba I

1 ATGGATGTTT ACAAGGAAGT TAATTTTCGTT GCTTACCTAC TAATTGTTCT  
 51 TGGTAAGATT TTCTTTTACT CTTTGTGTTT AAAAAATAAA AAAACAAAAA  
 101 AAATCTTGGT TTATACATAT ATATACACAC AAGTAGTTTT ATTTTTTTCC  
 151 TTTATATTAT ATTTGTTGTA GGAATATTTT TACTTGTTAG CGTGGTGGAA  
 201 CATGTTGATG CGAAGATCTG TACTAAAGAA TGTGGAATC TTGGGTTTGG  
 251 GATATGCCCA CGTTCAGAAG GAAGTCCGAA AAATCCCATG TGCATCAATT  
 301 GTTGCTCAGG CTATAAGGGT TGTAATTATT ATAGTGTGTT CGGGAGATTT  
 351 ATTTGCGAAG GAGAATCTGA CCTAAAAAC CCAAAAGCTT GCCCCCTAAA  
 401 TTGTGATACA AATATTGCCT ATTCAAGATG CCCCCATTCA GAAGGAAAAT  
 451 CGCTAATTTA TCCACCCGGA TGTACCACAT GTTGACACAG GTACAAGGGT  
 501 TGCTACTATT TCGGTAAAAA TGGCAAGTTT GTATGCCAAG GAGAGAGTGA  
 551 TGAACCCAAG GCAATATGT ACCCTGCAAT GTGA

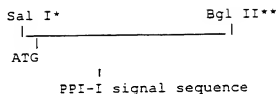
\* result of PCR error during isolation of the PPI-II sequence

Figure 4

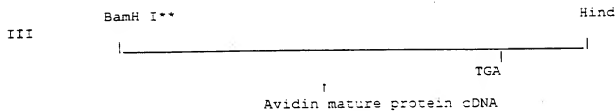
WO 00/04049

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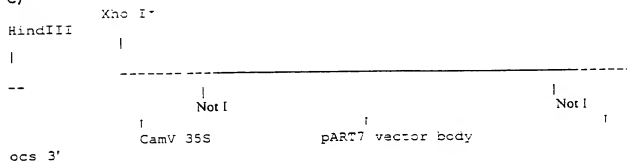
A)



B)



C)



\* compatible cohesive ends  
 \*\* compatible cohesive ends

Figure 5

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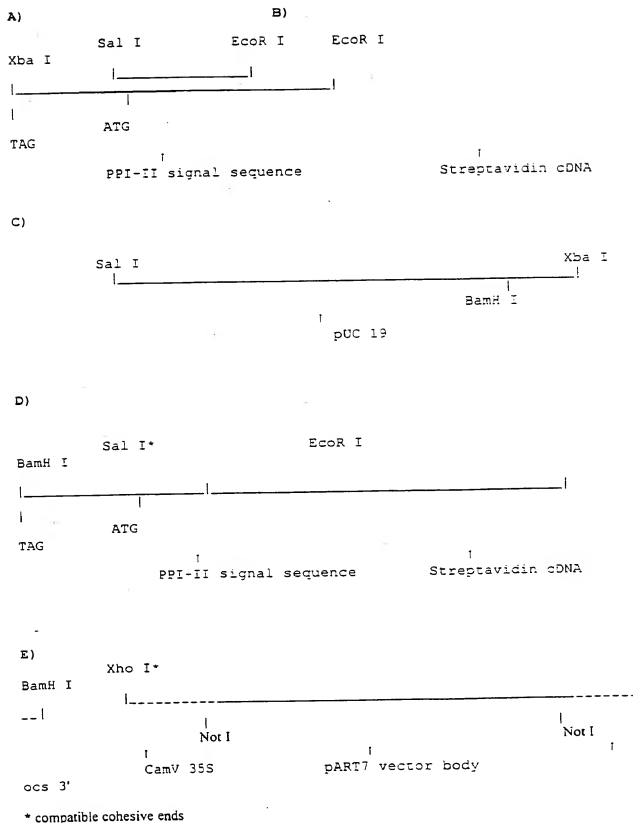


Figure 6

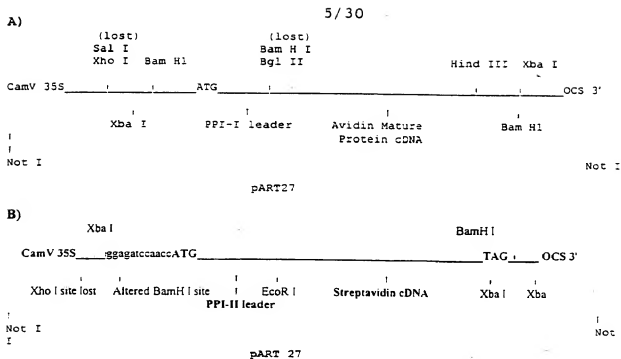


Figure 7

- A)
- 1 ATGGAGTCAA AGTTTGCTCA CATCATTGTT TTCTTTCTTC TTGCAACTCC  
51 CTTTGAAACT CTCTTGGCAC GAAAAGAAAG TGATGGACCA GAGATCCCTG  
101 CCAGAAAGTG CTCGCTGACT GGGAAATGGA CCAACGATCT GGGCTCCAAC  
151 ATGACCATCG GGGCTGTGAA CAGCAGAGGT GAATTCACAG GCACCTACAT  
201 CACAGCCGTA ACAGCCACAT CAAATGAGAT CAAAGAGTCA CCATTGCATG  
251 GGACACAAAA CACCATCAAC AAGAGGACCC AGCCACCTT TGGCTTCACC  
301 GTCAATTGGA AGTTTTCAGA GTCCACCACT GTCTTCACGG GCCAGTGCTT  
351 CATAGACAGG AATGGGAAGG AGGTCCTGAA GACCATGTGG CTGCTCGGCT  
401 CAAGTGTTAA TGACATTGGT GATGACTGGA AAGCTACCAG GGTGCGGCATC  
451 AACATCTTCA CTCGCCTGCG CACACAGAAG GAGTGA
- B)
- cleavage site  
↓
- 1 MESKFAHIIIV FLLATPFET LLARKESDGP EIPARKCSLT GKWTDNLGNS  
51 MTIGAVNSRG EFTGYIITAV TATSNEIKES PLHGTQNTIN KRTQPTFGFT  
101 VNWKFSESTT VFTGQCIFDR NGKEVLKTMW LLRSSEVNDIG DDWKATRVGI  
151 NIFTRLRTQK E\*

Figure 8

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A)

1 ATGGATGTTT ACAAGGAAGT TAATTCGTT GCTTACCTAC TAATTGTTCT  
 51 TGGTAAGATT TTCCTTTACT CCTTTGTTTT AAAAAATAAA AAAACAAAAA  
 101 AAATCTTGGT TTATACATAT ATATACACAC AAGTAGTTTT ATTTTTTTCC  
 151 TTTATATTAT ATTTGTTGTA GGAATATTTC TACTTGTTAG CGTGGTGGAA  
 201 CATGTTGATG CGAAGATCTG TACTAAGAAT TCGCATATGG CTGAAGCTGG  
 251 TATCACC GGT ACTTGGTACA ACCAGCTGGG GTCTACCTTC ATCGTTACCG  
 301 CTGGTGCTGA CGGTGCACTG ACCGGTACTT ACGAAAGCGC TGTGGTAAAC  
 351 GCTGAAAGCC GTTATGTTCT GACCGGTCGT TACGACTCTG CTCGGGCTAC  
 401 CGACGGTTCT GGTACTGCTC TGGGTTGGAC CGTTGCTTGG AAAACAACCT  
 451 ACCGTAACGC TCACTCTGCT ACCACCTGGT CTGGCCAGTA CGTTGGTGGT  
 501 GCTGAAGCTC GTATCAACAC CCAGTGGCTG CTGACCTCTG GTACCACCSA  
 551 AGCTAACGCT TGGAAATCTA CCTGGTGGT TCAACACAG TTCACCAAG  
 601 TTAACCGTC TGCTGCTTCT ATCTAG

B)

cleavage site

1 MDVHKVNFV AYLLIVLGIF LLVSVEHVD AKICTKshM AEAGITGTHY  
 51 NQLGSTFIPT AGADGALTGT YESAVGNAES RYVLTGRYDS. APATDGSCTA  
 101 LGWTVAWKNN YRNAHSATTW SGQYVGGAEA RINTQWLLTS GTTEANAKWS  
 151 TLVGHDTFTK VKPSAASI\*

Figure 9

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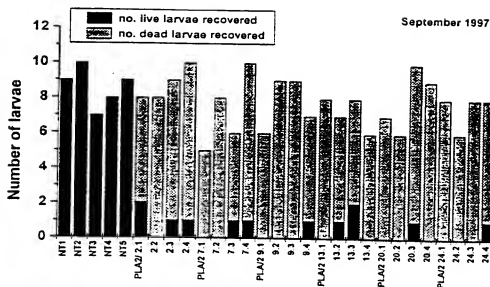


Figure 10

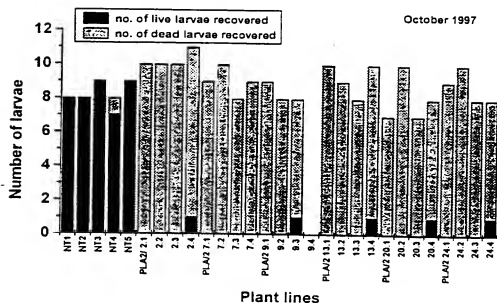


Figure 11

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A)

1 CCCTCCGTCC CGGCCGGSCA ACAACTAGGG AGTATTTTTC GTGTCTCACA  
51 TCGGCAAGAT CGTCGTTGCA GCCATCGCCG TTTCCTTGAC CACGGTCTCG  
101 ATTACGGCCA GCGCTTCGGC AGACCCCTCC AAGGACTCGA AGGCCCAGGT  
151 CTCGGCCGCC GAGGCCGGCA TCACCGGCAC CTGGTACAAC CAGCTCGGCT  
201 CGACCTTCAT CGTGACCGCG GCGGCCGACG GCGCCCTGAC CGGAACCTAC  
251 GAGTCGGCCG TCGGCAACGC CGAGAGCCGC TACGTCTTGA CCGGTCTTGA  
301 CGACAGCGCC CGGCCACCG ACGGCAGCGG CACCGCCCTC GGTGGACGG  
351 TGGCCTGGAA GAATAACTAC CGCAACGCCC ACTCCGCGAC CACGTGGAGC  
401 GGCCAGTACG TCGGCCGGCG CGAGGCGAGG ATCAACACCC AGTGGCTGCT  
451 GACCTCCGGC ACCACCGAGG CCAACGCCTG GAAGTCCACG CTGGTCCGGC  
501 ACGACACCTT CACCAAGGTG AAGCGTCCG CCGCCTCCAT CGACCGCGCG  
551 AAGAAGGCCG GCGTCAACAA CGGCAACCCG CTCGACGCCG TTCAGCAGTA  
601 GTCGCGTCCC GGCACCGGCG GGTGCCGGGA CCTCGGCC

B)

1 MRKIVVAAIA VSLTTSITA SASADPSKDS KAQVSAEAG ITGTWYNQLG  
51 STFIVTAGAD GALTGTYESA VGNAESRYVL TGRYDSAPAT DSGTALGWT  
101 VAWKNNYRNA HSATTWSGQY VGGAEARINT QWLLTSGTTE ANAWKSLVG  
151 HDTFTKVKPS AASIDAAKKA GVNNGNPLDA VQQ

Figure 12



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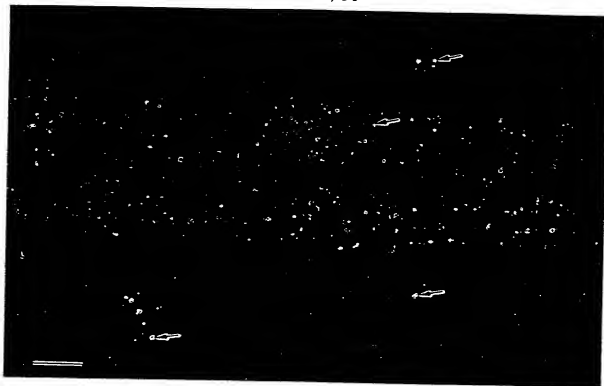


Figure 13

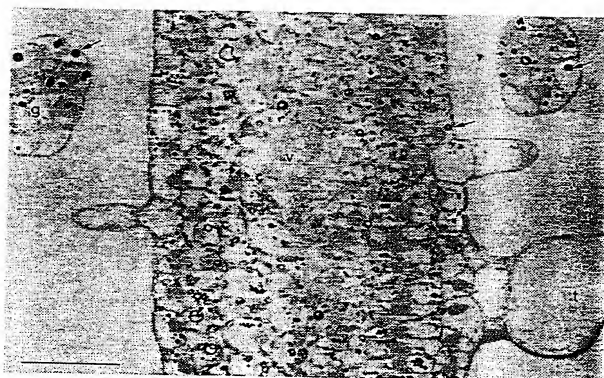


Figure 14

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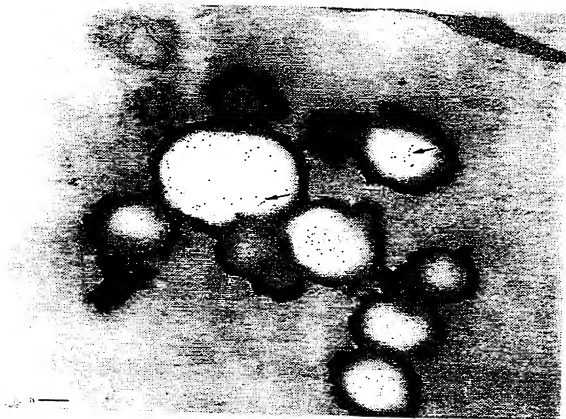


Figure 15

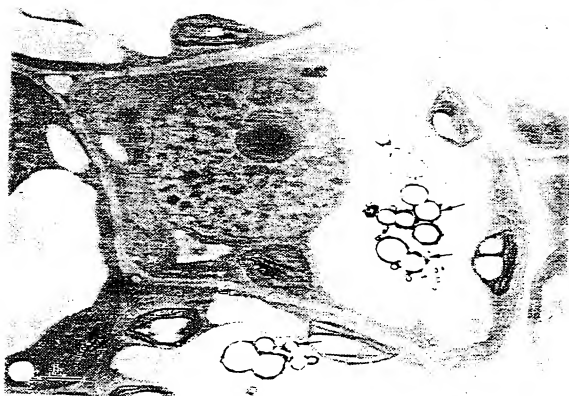


Figure 16

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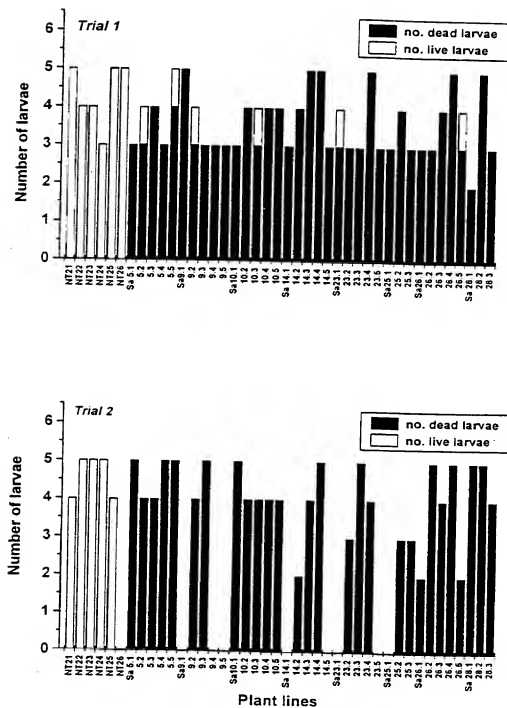


Figure 17

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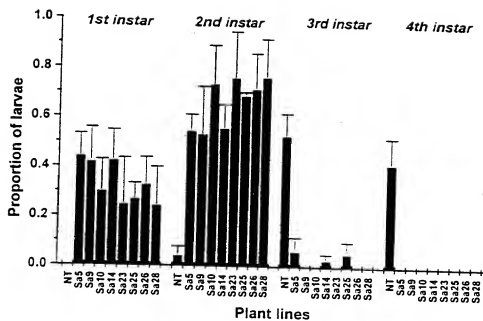


Figure 18

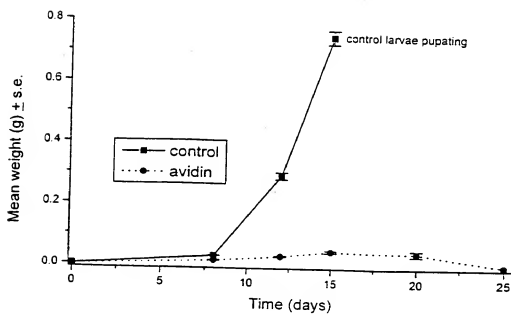


Figure 19A

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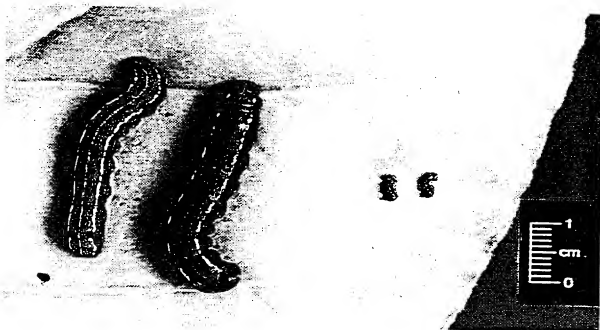


Figure 19B



Figure 19C

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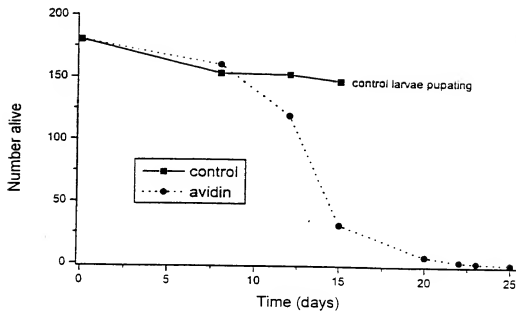


Figure 20

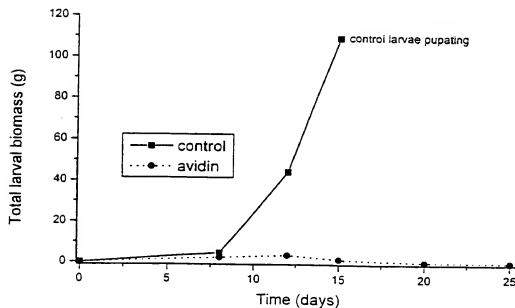


Figure 21

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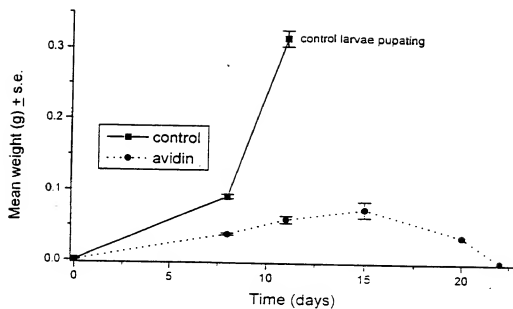


Figure 22A

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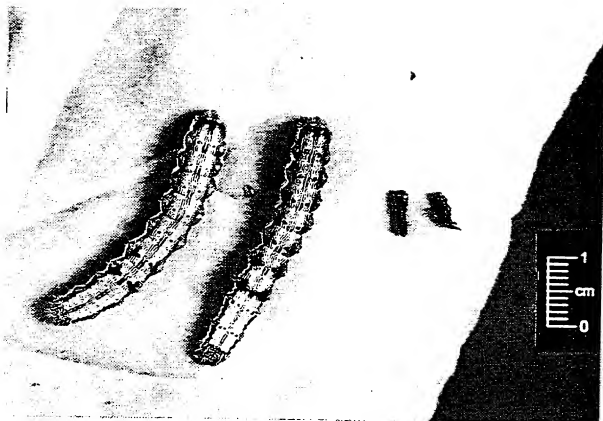


Figure 22B

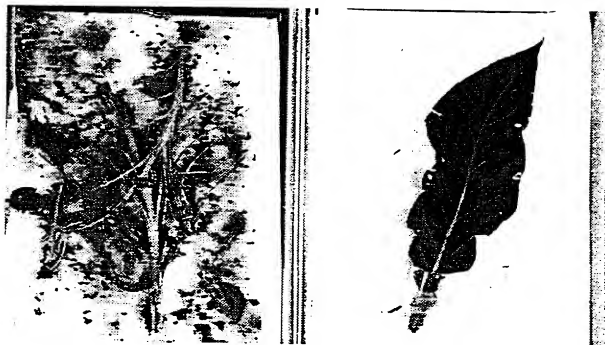


Figure 22C



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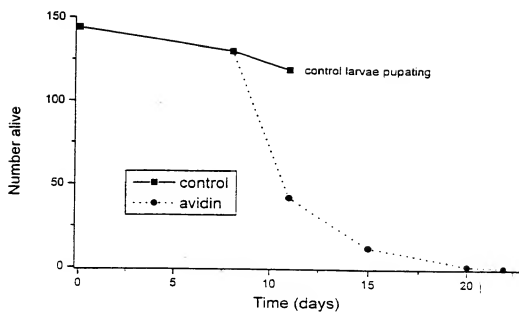


Figure 23

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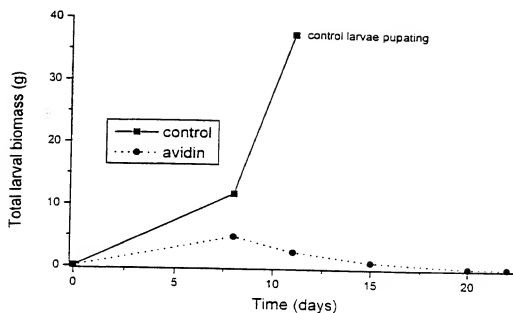


Figure 24

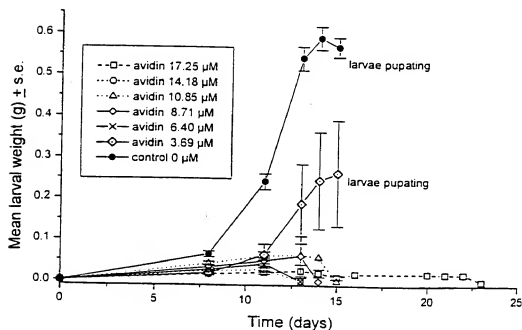


Figure 25

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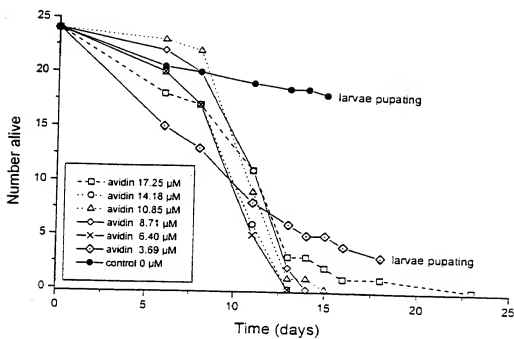


Figure 26

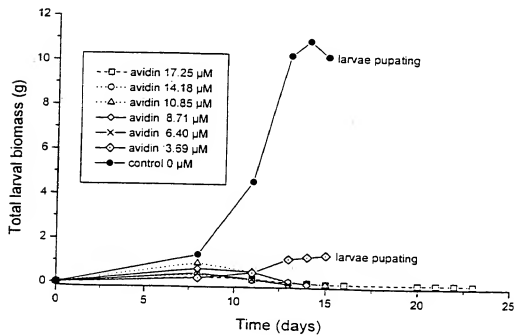


Figure 27

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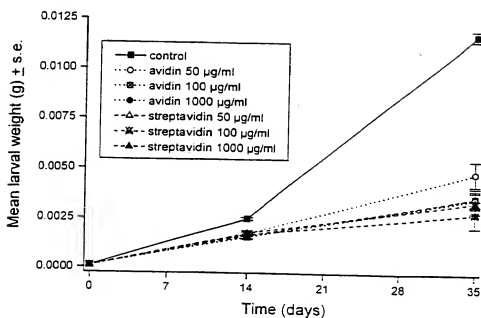


Figure 28

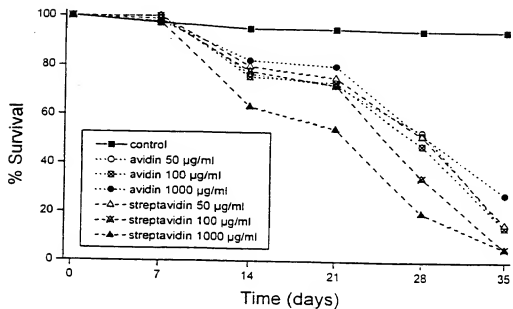


Figure 29

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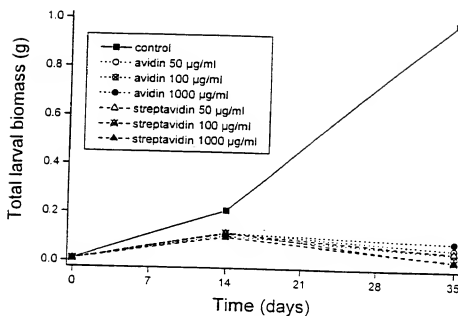


Figure 30

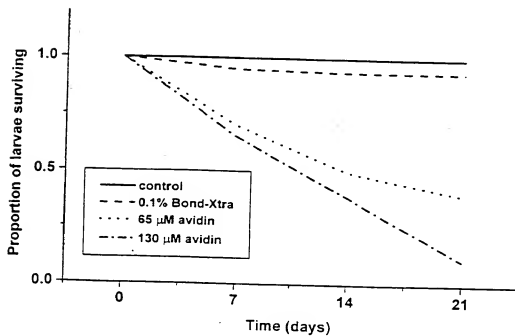


Figure 31

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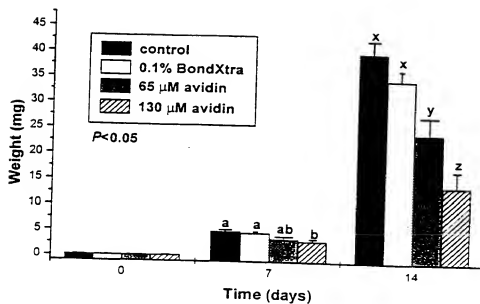


Figure 32

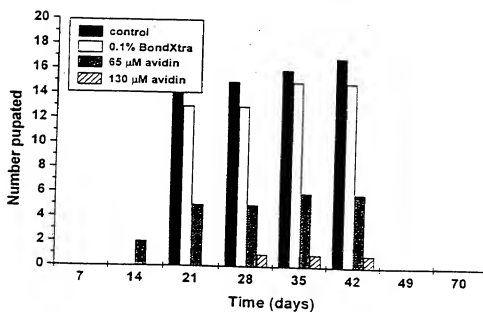


Figure 33

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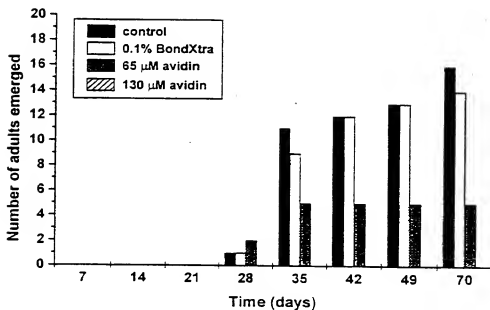


Figure 34

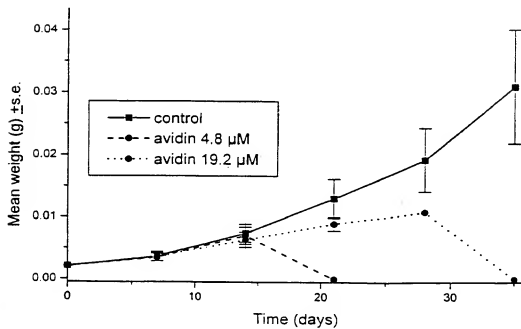


Figure 35

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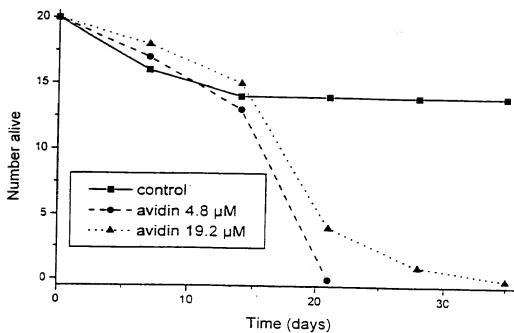


Figure 36

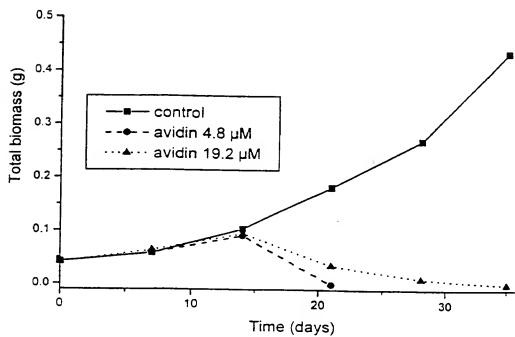


Figure 37



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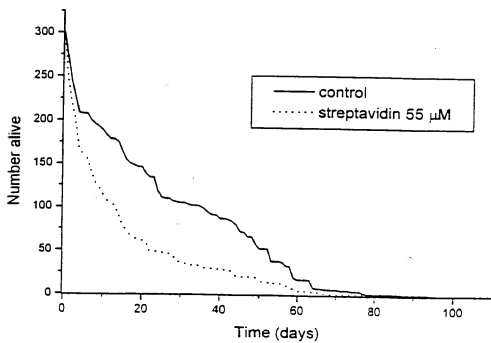


Figure 38

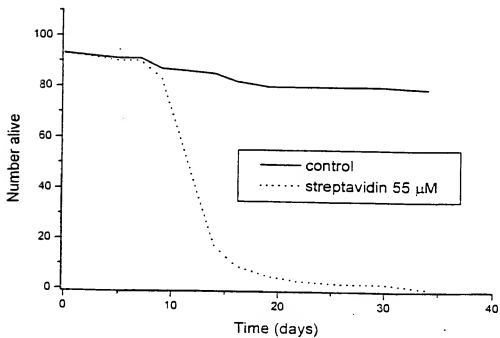


Figure 39

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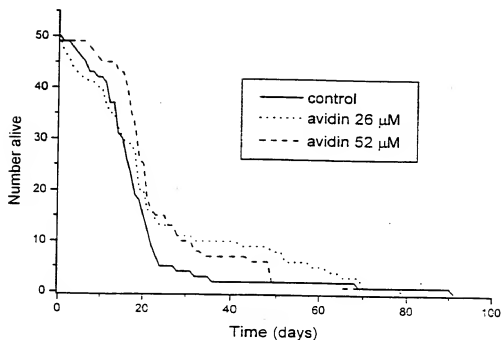


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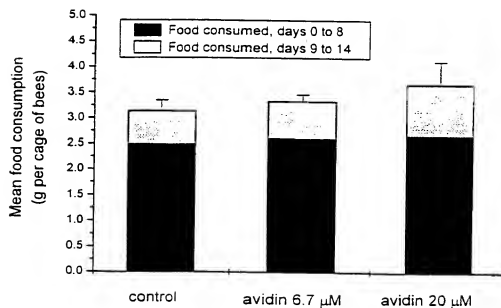


Figure 41

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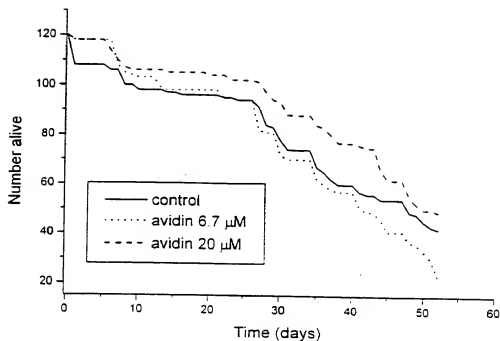


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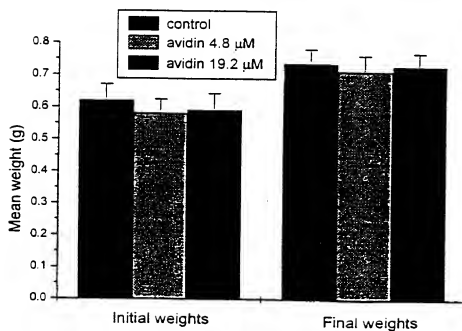


Figure 43

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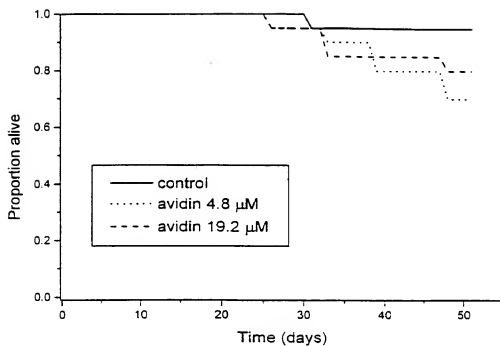


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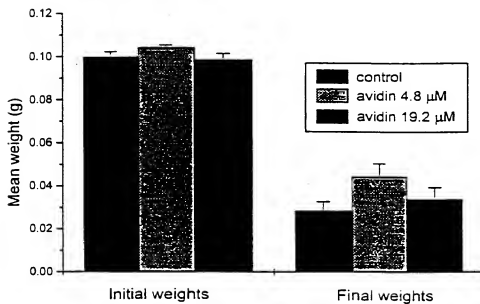


Figure 45

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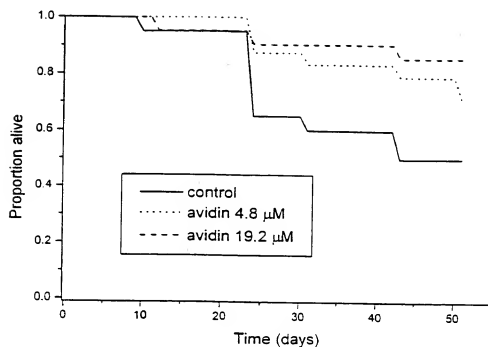


Figure 46

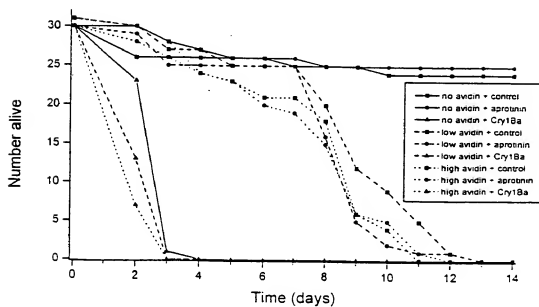


Figure 47

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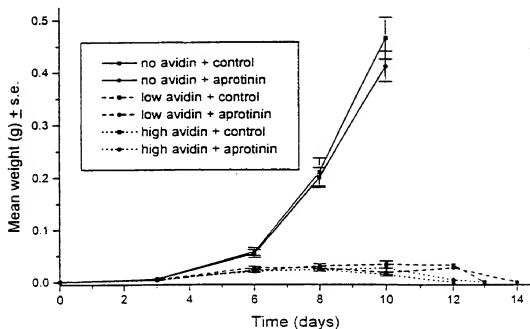


Figure 48

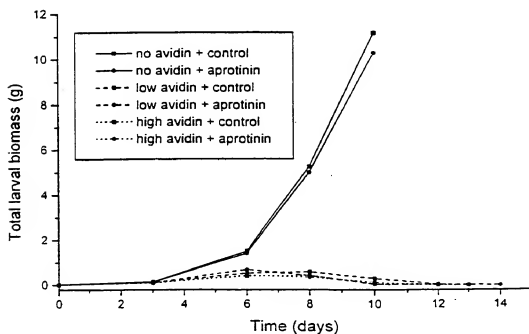


Figure 49